Field Efficacy of a Biopesticide Based on *Tithonia diversifolia* against Black Sigatoka Disease of Plantain (*Musa* spp., AAB)

Cécile Annie Ewané[1,2,3*], Robinson Nembot Tatsegouock[1,2], Arouna Meshuneke[1,2], Nicolas Niemenak[2,4]

[1]Laboratory of Phytoprotection and Plant Valorization, Biotechnology Center, University of Yaoundé 1, Yaoundé, Cameroon
[2]Laboratory of Plant Physiology, Higher Teacher's Training College, Yaoundé, Cameroon
[3]Department of Biochemistry, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon
[4]Department of Biological Science, Higher Teacher's Training College, Yaoundé, Cameroon
Email: *cecile-annie.ewane@facsciences-uy1.cm

How to cite this paper: Ewané, C.A., Tatsegouock, R.N., Meshuneke, A. and Niemenak, N. (2020) Field Efficacy of a Biopesticide Based on *Tithonia diversifolia* against Black Sigatoka Disease of Plantain (*Musa* spp., AAB). *Agricultural Sciences*, **11**, 730-743.
https://doi.org/10.4236/as.2020.118048

Received: July 25, 2020
Accepted: August 23, 2020
Published: August 26, 2020

Abstract

Black Sigatoka disease (BSD) is a foliar disease caused by *Mycosphaerella fijiensis*, responsible of reduction of the photosynthetic area of banana plant and yield at harvest since it has an influence on fruit physiology. The control of BSD relies on the use of chemicals which are not affordable for the small holder farmers and increase the cost of production. Moreover, this chemical control is ineffective, negatively impacting the environment and human health, and is at the origin of strain resistance. *Tithonia diversifolia* is known as rich in many compounds such as mineral elements, defense metabolites, some phytochemicals; and it is increasingly used in agriculture. Recently, the protective effect of *Tithonia diversifolia* liquid extract against BSD development on plantain vivoplants in the nursery was highlighted. The aim of our study was to evaluate the efficacy of a biopesticide base on *Tithonia diversifolia* on the BSD development in a plantain field under high disease pressure. The effect of *Tithonia diversifolia* biopesticide on *Mycosphaerella fijiensis* mycelial growth *in vitro* was evaluated. An experimental field at the flowering stage was selected and treated with the biopesticide base on *Tithonia diversifolia* at three different concentrations: undiluted (100%), diluted at 1/2 (50%) and diluted at 1/4 (25%) for 17 weeks. The disease severity, the number of functional leaves, the youngest spotted leaf (YSL) and the youngest necrotic leaf (YNL) were evaluated in course of time. The biopesticide treatments significantly ($P < 0.001$) reduce the BSD severity in course of time, but it is more effective for the most diluted concentration (25%). The number of leaves increases in course of time as well as the rank of the YSL and the YNL confirming the efficiency of BSD control. The efficacy of this biopesticide base on *Tithonia diversifolia* could be a hopeful ecoresponsible solution for the plantain sector in general and in particular for poor small farmers.

Keywords

Plantain (*Musa* spp.), Black Sigatoka Disease (BSD), *Mycosphaerella fijiensis*, *Tithonia diversifolia*, Biopesticide Efficacy

1. Introduction

Black Sigatoka disease is a foliar disease caused by the ascomycetous fungi *Mycosphaerella fijiensis* [1] [2]. It is the most economically important threat that the banana and plantain growing countries are facing. This parasitic fungus causes necrosis on the leaves and affects leaf photosynthesis, influencing directly the fruits filling and physiology, notably the fruit susceptibility to post-harvest diseases [3]. In severe cases, a substantial reduction of the leaf area is observed, leading to reduction in yield at harvest with loss greater than 50% of production [4]. This loss is critical since plantain is a crop which is rich in nutrients, playing a vital role in contributing to food security for the people in the worldwide.

Mycosphaerella fijiensis was effectively reported for the first time in Cameroon in 1980 and it has totally replaced *M. musicola* [5]. The climatic conditions (warm temperature and high rainfall) of this agroecosystem provide a suitable environment for spore's proliferation, multiplication and spreading. The susceptibility of bananas to BSD depends on many preharvest factors such as the type of banana cultivar used, the growth physiological stage of the plant (cycle period), the type of soil used, the season... Indeed, most banana cultivars grown for exportation are highly susceptible to BSD, while many of the plantains cultivars (AAB) and the AAA, AAB, ABB cooking bananas are highly or moderately susceptible to BSD [6] [7]. In addition, the less susceptibility to BSD was found in cooking bananas than plantains and sweet bananas, before flowering than after flowering periods, and in cultivation sites rich in organic matter compared to the poor one [8].

The main control methods currently used consist of mechanical deleafting of disease leaves and the application of fungicides which relies on aerial applications according to either systematic frameworks *i.e.* mostly contact fungicides or forecasting strategies *i.e.* mostly systemic fungicides [9]. However, the mechanical deleafting is not effective during the high-pressure period (rainy season) and the use of fungicides has led to the rapid emergence of fungicide resistance, conducive to a significant increase in the cost of disease control but, above all, to increasing negative environmental and health effects [9]. The chemical control of BSD requires regular application of fungicide treatments that had an important economic impact through the increase of the production costs not affordable by poor smallholder farmers and householders.

One of the banana production chain challenges is a limitation of the fungicide usage in BSD control, and it requires the use of a low cost and ecoresponsible biopesticide as an interesting alternative to poor small holder farmers. *T. diversifolia* biopesticide could be a solution to this problem since it is both less restrictive for the producer, and preserver of the environment and human health. Indeed, it contains many minerals and some secondary metabolites like alkaloids, flavonoids and terpenoids [10] [11] [12]. Moreover, recent studies have highlighted the fertilizer, the protective, the insecticide and the fungicide effects of aqueous extract of *T. diversifolia* on different plants [13] [14]. It has been recently shown that *Tithonia diversifolia* liquid extract used to water plantain vivoplants in the nursery was having a growth promotion action as well as a protective action against BSD [15]. A biopesticide base on *Tithonia diversifolia* has been formulated despite no knowledge was available about the minimal effective concentration to use. The purpose of our study was therefore to evaluate the efficacy of a biopesticide base on *Tithonia diversifolia* on the development of the BSD in a plantain field under high disease threat.

2. Materials and Methods

2.1. Materials

Plantain plants grown in the peasant farm constituted of the plantain variety Big-Ebanga was used for this experimentation from December 2018 to March 2019 (17 weeks). The plantain plants were selected at the flowering stage (about 6 months ages) of the first cycle of production and were under a high black Sigatoka disease (BSD) severity threat of about 45% to 47%.

The peasant farm was located in Nkoabang (Yaoundé) in the 4^{th} district of the city of Yaoundé (North-East), in the Centre-Cameroon region. Its geographical coordinates are: 3˚40'60'' North latitude and 11˚25'0'' East longitude. This Centre region is located in the agro-ecological zone known as humid forest with bimodal rainfall, altitude 714 m, average temperature 23.7˚C, mean annual rainfall 1643 mm. Therefore, its climatic conditions provide a favourable environment for the development of BSD and a high BSD pressure has been observed throughout the surroundings area.

The biopesticide based on *T. diversifolia* was used at different concentrations: undiluted (100%), diluted with water at 1/2 (50%) and at 1/4 (25%).

The plantain farm was separated in three blocks (Block A, Block B and Block C) of ten plants each and they were treated once a week with three different concentrations of the biopesticide. According to the level of severity of BSD and proximity between the plants, mechanical deleafting of disease leaves was done on the plot only at the beginning of the experimentation.

2.2. Confrontation Test of *Mycosphaerella fijiensis* and *T. diversifolia* Biopesticide

The growth of *Mycosphaerella fijiensis* in the presence of biopesticide based on *T. diversifolia* was done *in vitro*. *M. fijiensis* was cultured on a PDA media (potatoes-dextrose-agar) in the presence of the biopesticide added by well diffusion. Two wells of 5 mm in diameter were made at 2 cm on either extreme side of the Petri dish culture and the *M. fijiensis* strain placed in the middle while 10 μL of the biopesticide was added in the wells (**Figure 1**). The biopesticide was replaced with sterilized distilled water in the wells of the control Petri dishes. The cultures were subsequently incubated in the light at room temperature. The diameter of inhibition of growth was evaluated by measuring the zone of inhibition every 3 days on the side of the fungal culture and compared with the growth of the control dishes [16].

2.3. Evaluation of the Disease Severity

BSD severity was observed and evaluated on all selected plantain plants at the flowering stage in order to characterize the intensity of necrotic leaf area. The estimation of the percentage of necrotic leaf area per plant through the Severity Index (SI) calculation was carried out according to the method described by [17]:

$$SI = \left[\left(\sum \text{scores}/6\right) \times \text{NTL}\right] \times 100$$

Σscores are the sum of all infestation indices of the plantain plant.

NTL is the number of leaves of the plantain plant.

This method enables the scoring of the severity index of each leaf according to the following scale:

0: no necrotic lesions;

1: less than 1% necrotic lesions;

2: 1% - 5% necrotic lesions;

3: 6% - 15% necrotic lesions;

4: 16% - 33% necrotic lesions;

5: 34% - 50% necrotic lesions;

6: more than 50% necrotic lesions.

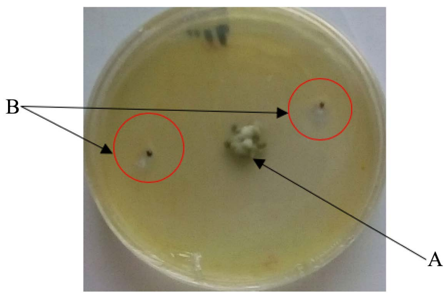


Figure 1. Confrontation test in a Petri dish showing a culture of *Mycosphaerella fijiensis* (A) strain aged of thirty days grown on PDA medium in the presence of sterilized distilled water (control), put in the wells (B) for diffusion in the media.

2.4. Evaluation of the Efficiency of BSD Control with the Biopesticide Based on *T. diversifolia*

In order to characterize the efficiency of BSD control method [9], the number of functional leaves, the youngest spotted leaf and the youngest necrotic leaf were assessed. The number of functional leaves was evaluated to characterize the photosynthetic potential of the plantain tree and it was determined with the assumption that functional leaves should have less than 30% of necrotic surface, *i.e.* an infestation index of the leaf < 5.

The youngest spotted leaf (YSL) and the youngest necrotic leaf (YNL) from the flowering to the end of the experimentation was assessed according to the method of [18], which involves the monitoring of the youngest leaf (from the top of the plant) bearing at least 10 necrotic lesions at stage 1 and the monitoring of the youngest leaf (from the top of the plant) bearing 50% necrotic lesions at stages 5 and 6 respectively.

2.5. Statistical Analysis

The effect of the biopesticide based on *Tithonia diversifolia* on the mycelial growth of *M. fijiensis* was subjected to fixed two-way ANOVA performed with XLSTAT software [19], with "treatment" and "time" as factors. The effect of the different biopesticide concentrations on the percentage of BSD severity, the number of functional leaves, the youngest leaf spotted and on the youngest leaf necrosed from the flowering stage to the end of the experimentation were analysed by the Microsoft Excel 2019. Separations of means were based on Tukey's multiple range tests at a 5% probability level.

3. Results

3.1. Confrontation Test of *Mycosphaerella fijiensis* and the Biopesticide Based on *T. diversifolia*

The mycelial growth of *Mycosphaerella fijiensis in vitro* is significantly ($P <$ 0.0001) influenced by the presence of the biopesticide based on *Tithonia diversifolia*. The treatment, the time and the interaction between the treatment and time were highly significant ($P < 0.0001$) as shown in **Table 1**. The coefficient of determination R^2 was close to a 100% (**Table 1**) and the most influential variable was the time.

Table 1. Variance analysis of the *in vitro* mycelial growth of *Mycosphaerella fijiensis* culture in a confrontation by well diffusion on PDA media in the presence of *Tithonia diversifolia* biopesticide.

Source	DF	F	*P*
Treatment	1	67,340.182	**<0.0001**
Time (Days)	13	51,205.657	**<0.0001**
Treatment * Time (Days)	13	4193.442	**<0.0001**

Values in bold correspond to tests where the null hypothesis is not accepted with a significance level alpha = 0.05. DF is the degree of freedom; *F* is the value of F test and *P* is the probability.

The growth of *M. fijiensis* in the presence of the biopesticide was observed 12 days after transplanting the strain in the Petri dishes containing PDA media. The kinetic of mycelial growth evolution was marked by a latency phase of approximately 9 days, followed by an exponential growth phase until the 18th day for the confronted and 27 days for the control, then has intervene a growth slowdown in the dishes (**Figure 2(a)**) until the end of the follow-up period. A significant difference was observed between the mycelial growth of the confronted *Mycosphaerella fijiensis* culture and the control one (**Figure 2(b)**). The presence of the biopesticide based on *Tithonia diversifolia* in the Petri dish inhibits the mycelial growth of *Mycosphaerella fijiensis*. This mycelial growth was two times more important in the control culture compared to the confronted one after 39 days of culture on PDA media in Petri dishes as confirmed by the two statistical distinct groups obtained.

3.2. Evaluation of the BSD Severity

The BSD severity was almost the same in the three experimental blocks at the beginning of the experiment (week 1) and has decreased gradually in course of time till week 17. The kinetic of BSD severity decrease is not the same between the blocks. The undiluted extract (100% biopesticide) reduces the severity of BSD slowly compare to the most diluted (25% diluted biopesticide) treatment (**Figures 3(a)-(c)**). The treatment effect on the reduction of the plantain plant BSD severity was noticed after one week of treatment, but was more effective in the blocks where the plantain plants were treated with diluted biopesticide (50% and 25%) compare to the one treated with the undiluted (100%) biopesticide (**Figure 3**).

The mean percentage of BSD severity decreases from 44.6%, 45.9% and 47% to 32.9%, 25.6% and 19.9% after one week respectively for biopesticide based on *Tithonia diversifolia* treatment applied at 100% (Block 1), 50% (Block 2) and 25% (Block 3) concentrations. The BSD severity was gradually decreasing in

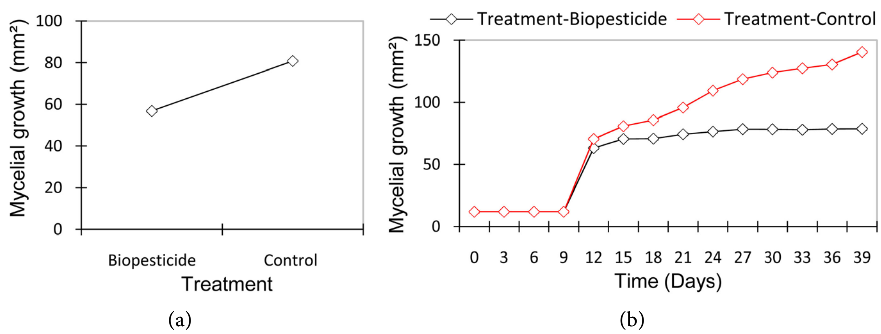
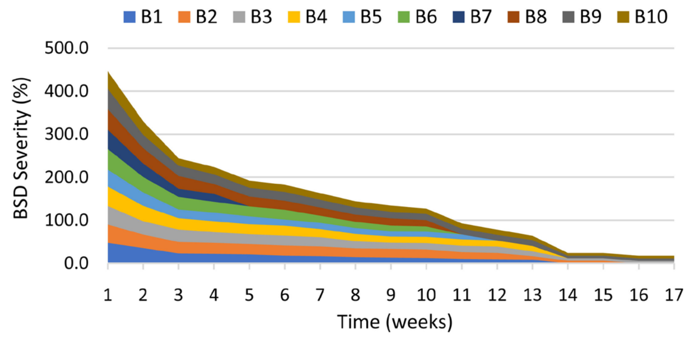
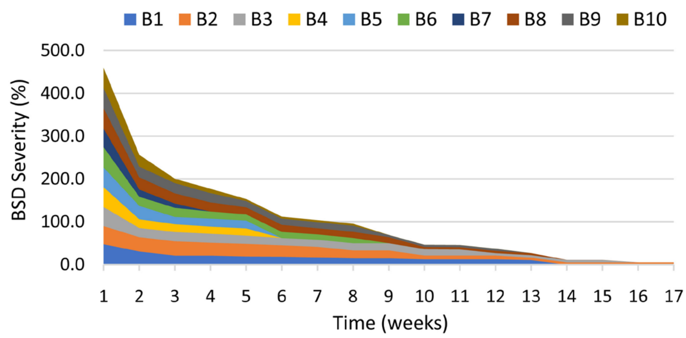


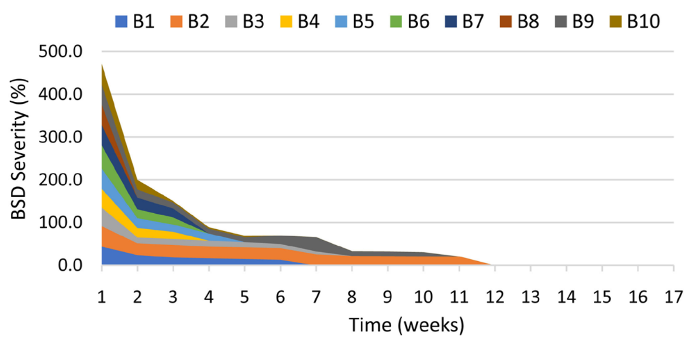
Figure 2. Effects of the undiluted biopesticide (100%) based on *Tithonia diversifolia* on the *in vitro* mycelial growth of *Mycosphaerella fijiensis* culture confronted by well diffusion on PDA media. Plot of means treatments (a); Interaction plots of time (day) and treatment (b) for mycelial growth. Each point represents the average mean of three replicates with the standard deviation for each treatment.



(a)



(b)



(c)

Figure 3. BSD severity evolution stage in percentage (%) during the seventeen (17) weeks assessment for each plantain plant (experimental unit) in the field for the third blocks: (a) 100% undiluted treatment; (b) 50% diluted treatment and (c) 25% diluted treatment.

course of time and was a 100% effective in Block 3 (25%) after eleven weeks of treatment, while it was still present after seventeen weeks of treatment in Block 1 (100%) and Block 2 (50%) with a more important severity at that date exhibited by plantain plants of Block 1 (Figure 4). Despite the return of rains from week 11, the level of BSD severity remains low in the three blocks till the end of the experimental period.

3.3. Evaluation of the Efficiency of BSD Control with the Biopesticide Based on *Tithonia diversifolia*

The mean number of functional leaves increases gradually in course of time and it is almost the same in the three treatment blocks as shown in Figure 5 from the

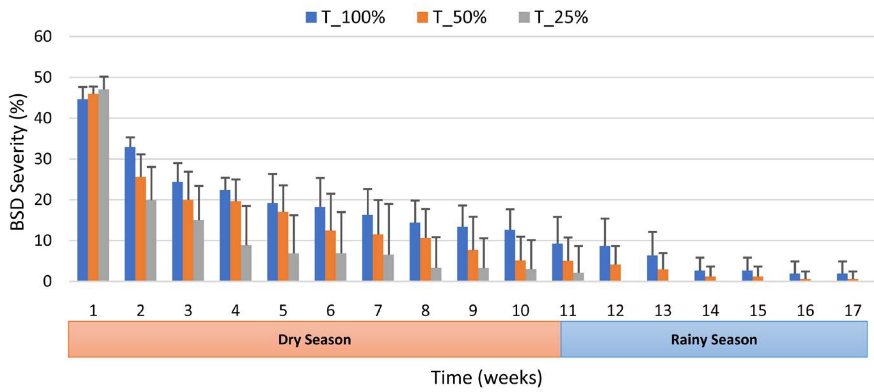


Figure 4. BSD severity evolution stage in percentage (%) during the seventeen (17) weeks assessment for each treatment block (100% undiluted, 50% diluted and 25% diluted treatments) in the field. Each point represents the average mean of ten replicates with the standard deviation for each treatment.

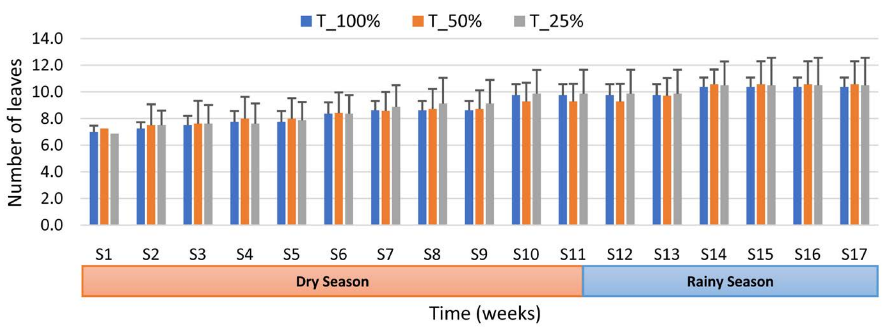


Figure 5. Evolution of the number of functional leaves during the seventeen (17) weeks assessment for each treatment block (100% undiluted, 50% diluted and 25% diluted treatments) in the field. Each point represents the average mean of ten replicates with the standard deviation for each treatment.

start to the end of the experimentation. The interaction plot between the evolution of the BSD severity and the number of functional leaves clearly shows that when the number of leaves increases, the BSD severity decreases with an interception point differing according to the treatment blocks (**Figure 6**). The interception between both curves occurs very early for the 25% diluted biopesticide treatment, followed by the 50% diluted biopesticide treatment and the 100% undiluted biopesticide treatment; that is at five days, nine days and eleven days respectively.

The evolution of the youngest spotted leaf (YSL) showed a reduction in the rank of the spotted leaf in course of time (week 1 to week 17) from leaf 3 to leaf 9 in the 50% and 100% biopesticide treatment, while in the 25% biopesticide treatment it goes from week 1 to week 13 and reduces from leaf 3 to leaf 7 (**Figure 7**). The evolution of the youngest necrotic leaf (YNL) also showed a reduction in the rank of the necrotic leaf in course of time in the three blocks treatments from week 1 to week 7 with no necrosis at week 4 and week 6, and no spotted leaf from week 8 to week 17 (**Figure 8**). Despite the return of rains, the

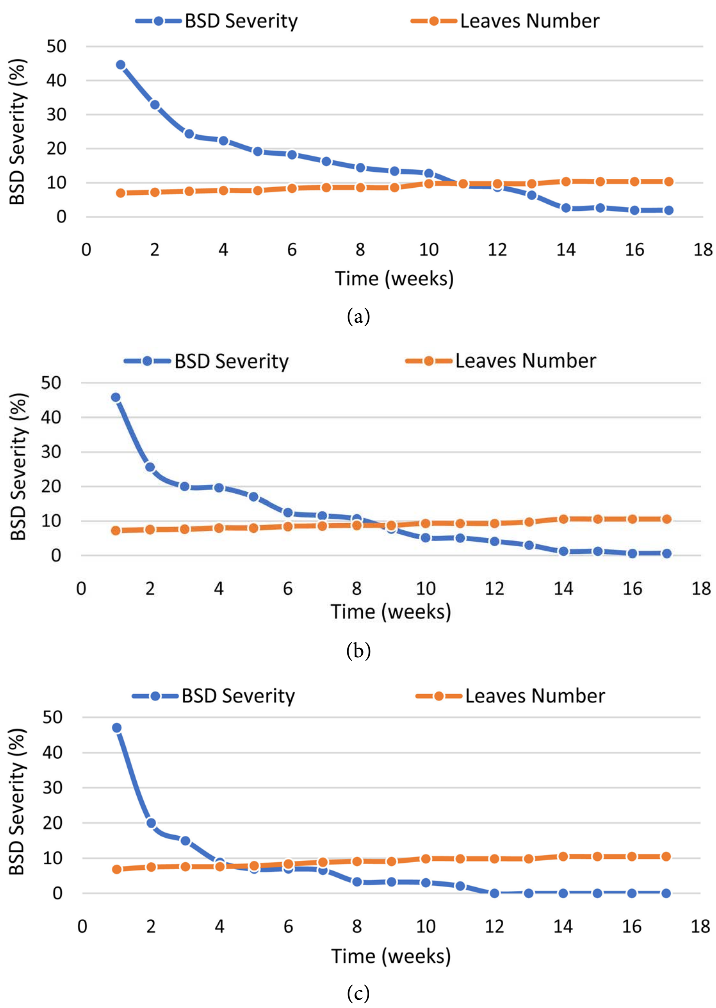


Figure 6. Interaction plot between the evolution of the BSD severity and the number of functional leaves during the seventeen (17) weeks assessment: (a) 100% undiluted treatment; (b) 50% diluted treatment and (c) 25% diluted treatment.

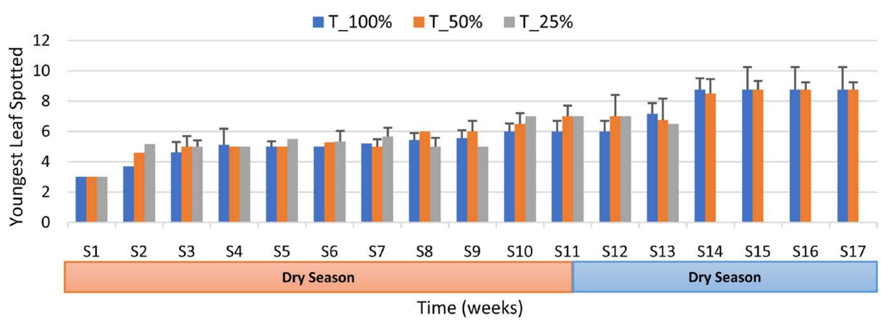


Figure 7. Evolution of the youngest spotted leaf during the seventeen (17) weeks assessment for each treatment block (100% undiluted, 50% diluted and 25% diluted treatments) in the field. Each point represents the average mean of ten replicates with the standard deviation for each treatment.

YSL and the YNL were not increased in the three blocks biopesticide treatments till the end of the experimental period.

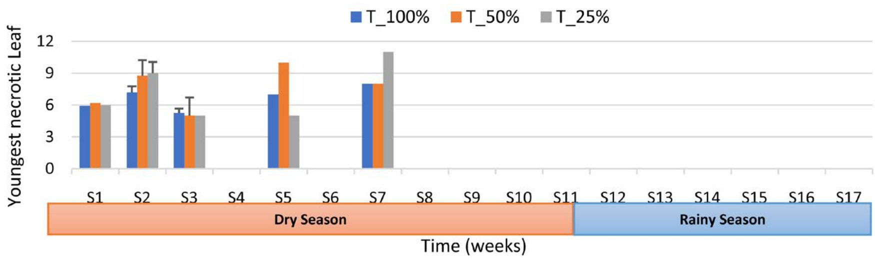


Figure 8. Evolution of the youngest necrotic leaf during the seventeen (17) weeks assessment for each treatment block (100% undiluted, 50% diluted and 25% diluted treatments) in the field. Each point represents the average mean of ten replicates with the standard deviation for each treatment.

4. Discussion

The biopesticide based on *T. diversifolia* influences significantly the *in vitro* mycelial growth of *Mycosphaerella fijiensis* on PDA media compared to the control culture. An inhibition of about 50% was observed in the development of *M. fijiensis* and the growth was only observable from the 12th day after transplanting. Subsequently, especially on the 18th day, we observed a slowdown in the development of *M. fijiensis* in the presence of the biopesticide, which is in agreement with [4] who noted that the culture of *M. fijiensis* was very slow, averaging 1 cm after 38 days. These results also suggested that the biopesticide diffused slowly to establish itself in the Petri dish in order to induce its bioactivity, confirming the fungistatic effect of the secondary metabolites contained in the biopesticide based on *T. diversifolia* on the mycelial growth rate in agreement with [14] who had shown that extracts of *T. diversifolia* were rich in tannins and sesquiterpenes.

The biopesticides based on *T. diversifolia* applied at different concentrations (100%, 50% and 25% blocks) reduce considerably the BSD severity in the three blocks in field. The most effective biopesticide treatment was in the most diluted one (25%, Block 3) compared to the others, suggesting thus that the most the biopesticide was diluted, the most it was effective. This result revealed an effective antifungal activity with different kinetic of diseases evolution depending of the biopesticide treatment concentration applied (100%, 50% and 25% blocks) and corroborated with previous researches that have assigned fungicidal and/or fungistatic, insecticidal, repulsive and antimicrobial activities to *T. diversifolia* extracts [12] [14] [15] [20] [21]. However, it would be interesting for more efficiency to assessed and determine the minimum effective dose of this biopesticide *in vitro* and *in vivo*.

The less severity observed on the plantain blocks in the field after the biopesticide treatments could be due to a direct effect through the formation of an antagonistic microorganisms and/or secondary metabolites repulsive film on the plant leaves, or an indirect effect due to the establishment of a local and an induced resistance. Indeed, *T. diversifolia* contribute to plant protection by modifying the microbial community and stimulating plant defense mechanisms [14].

Moreover, this biopesticide based on *T. diversifolia* contains many minerals and secondary metabolites such as nitrogen, phosphorus, potassium, magnesium, proteins and phenolic compounds (alcaloids, sesquiterpens, lactones and flavonoids), involved in different physiological role in the plant [10] [11] [12]. In addition, defensive metabolites have been shown acting on the susceptible *Musa* spp. plant as biofungicide against BSD [8]. It would be therefore interesting to assess the biochemical and microbial composition of this biopesticide based on *T. diversifolia*.

The efficacy of the biopesticide based on *T. diversifolia* treatment has been shown by the less rank of indicators commonly used to characterize the efficiency of control methods; that is the youngest spotted leaf and the youngest necrotic leaf in the three blocks (100%, 50% and 25%). Recently, the treatment of plantain vivoplants with *T. diversifolia* biopesticide in the nursery has revealed the induced resistance against BSD through the high accumulation of the growth promotion and defense biomarkers in the leaves of treated plants [21], but none was known about the mode of action of this extract when it is spread on the leaves. It would be therefore interesting to assess the mode of action of this biopesticide based on *T. diversifolia* on the leaves' tissues.

The number of functional leaves was increased thanks to the biopesticide based on *T. diversifolia* treatment during the experimental period and it suggests the good photosynthetic potential of the treated plantain plant, especially for the 25% diluted treatment block. This result revealed less stress due to BSD severity, the good assimilation and metabolites accumulation leading to fruit filling conditions that are impacting positively the yield. Our results are in line with previous studies that have revealed that the high BSD severity induces the high reduction in the photosynthetic area that impact on fruit morphology and inversely [3] [22] [23] [24] [25]. Moreover, *T. diversifolia* are rich in mineral elements such as nitrogen, deeply involved in plant growth promotion; its content and form play an essential role in defensive primary and secondary metabolism and NO-mediated events [26].

The excessive assimilates accumulation in this condition can contributes to the important energetic demand for secondary metabolic pathways that promote the development of defense mechanisms as hypothesized in the case of the susceptibility of banana fruits to crown rot [25]. Indeed, in the case of plantain seedlings in the nursery, it has been recently shown that the seedlings treatment with *Tithonia diversifolia* based products is inducing the growth promotion as well as the plant protection against *Mycosphaerella fijiensis* [21] [27] [28] [29], suggesting thus a link between the growth promotion and the less susceptibility to disease by treated plants in the nursery and the field.

5. Conclusion

To sum up, our study highlighted a new effect of biopesticide based on *T. diversifolia* on BSD severity in the field. It would be however interesting to assess seasonal and spatiotemporal variation of this biopesticide effect on BSD severity and the physiological mechanisms involved in its efficiency. The biopesticide based on *T. diversifolia* should also be characterized, applied on other pathosystems and a product developed for the preservation of the human health and the environment, as well as small holders' farmers empowerment.

Acknowledgements

The authors wish to thank M. Sylvestre Matip owner of the experimental field for his collaboration on this project. Gratitude especially goes to the dedicated technical assistance of Fabrice Damien Wassom and Gaston Elock Mbang in this project.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Jones, D.R.D. (2000) Diseases of Banana, Abaca and Enset. CABI Publication, Wallingford, 544 p.

[2] Lassoudière, A. (2007) Le bananier et sa culture. Editions Quae, 383 p.

[3] Ewané, C.A., Chillet, M., Castelan, F., Brostaux, Y., Lassois, L., Ngando, E.J., Hubert, O., Chilin-Charles, Y., Lepoivre, P. and de Lapeyre de Bellaire, L. (2013) Impact of the Extension of Black Leaf Streak Disease on Banana Susceptibility to Post-Harvest Diseases. *Fruits*, **68**, 351-365. https://doi.org/10.1051/fruits/2013081

[4] Onautshu, O.D. (2013) Caractérisation des populations de *Mycosphaerella fijiensis* et épidémiologie de la cercosporiose noire du bananier (*Musa* spp.) dans la région de Kisangani-République Démocratique du Congo. Thèse de doctorat ès science, Université Catholique de Louvain.

[5] Fouré, E. and Lescot, T. (1988) Variabilité génétique des *Mycosphaerella* inféodés au genre *Musa*. Mise en évidence de la présence au Cameroun sur bananier et plantain d'une cercosporiose (*Mycosphaerella musicola*) au comportement pathogène atypique, *Fruits*, **43**, 407-415.

[6] Gowen, S. (2012) Bananas and Plantains. Springer Science & Business Media, Berlin, 612 p.

[7] Bennett, R.S. and Arneson, P.A. (2003) Black Sigatoka. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2003-0905-01

[8] Oluma, H.O.A., Onekutu, A. and Onyezili, F.N. (2004) Reactions of Plantain and Banana Cultivars to Black Sigatoka Leaf Spot Disease in Three Farming Systems in the Nigerian Guinea Savanna. *Journal of Plant Diseases and Protection*, **111**, 158-164. https://doi.org/10.1007/BF03356142

[9] de Lapeyre de Bellaire, L., Fouré, E., Abadie, C. and Carlier, J. (2010) Black Leaf Streak Disease Is Challenging the Banana Industry. *Fruits*, **65**, 327-342. https://doi.org/10.1051/fruits/2010034

[10] Oyerinde, R.O., Otusanya, O.O. and Akpor, O.B. (2009) Allelopathic Effect of *Tithonya diversifolia* on the Germination, Growth and Cholorophyl of Maize (*Zea*

mays L.). *Scientific Research and Essay*, **4**, 879-888.

[11] Kaho, F., Yemefack, M., Feudjio-Teguefouet, P. and Tchantchouang, J.C. (2011) Effet combiné des feuilles de *Tithonya diversifolia* et des engrais inorganiques sur les rendements du maïs et les propriétés d'un sol ferralitique au Centre Cameroun. *Tropicultura*, **29**, 39-45.

[12] Kerebba, N., Oyedeji, A.O., Byamukama, R., Kuria, S.K. and Oyedeji, O.O. (2019) Pesticidal Activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); Phytochemical Isolation and Characterization: A Review. *South African Journal of Botany*, **121**, 366-376. https://doi.org/10.1016/j.sajb.2018.11.024

[13] Kandungu, J., Anjarwalla, P., Mwaura, L., Ofori, D.A., Jammadass, R., Stevenson, P.C. and Smith, P. (2013) "Pesticidal Plant Leaflet" *Tithonya diversifolia* (Hemsley) A. Gray. Kew Royal Botanic Gardens, World Agroforestry Centre.

[14] Diby, Y.K.S., Tahiri, Y.A., Akpesse, A.A.M., Trabi, C.S. and Kouassi, K.P. (2015) Evaluation de l'effet insecticide de l'extrait aqueux de Tit*honia diversifolia* (Hemsl.) gray (*Asteracee*) sur les termites en culture du riz (NERICA 1) au centre de la Cote d'Ivoire. *Journal of Animal & Plant Sciences*, **25**, 3966-3976.

[15] Tatsegouock, R.N., Ewane, C.A., Meshuneke, A. and Boudjeko, T. (2020) Plantain Bananas PIF Seedlings Treatment with Liquid Extract of *Tithonia diversifolia* Induces Resistance to Black Sigatoka Disease. *American Journal of Plant Science*, **11**, 653-671. https://doi.org/10.4236/ajps.2020.115049

[16] Dihazi, A. (2012) Interaction palmier dattier-*fusarium oxyporium* f. sp albedinis: Induction des réactions de défense par l'acide salicylique et rôle de quelques microorganismes antagonistes de l'agent pathogènes dans le contrôle de la maladie de Bayoud. Thèse présentée en vue de l'obtention d'un Doctorat National. Laboratoire de biotechnologie et de valorisation des ressources naturelles, Faculté des sciences, Agadir.

[17] Gauhl, F., Pasberg-Gauhl, F., Vuylsteke, D. and Ortiz, R. (1993) Multilocational Evaluation of Black Sigatoka Resistance in Banana and Plantain, IITA, IITA Res. Guide No. 47, Ibadan, 59 p.

[18] Stover, R.H. and Dickson, J.D. (1976) Banana Leaf Spot Caused by *Mycosphaerella musicola* and *M. fijiensis* var. Difformis: A Comparison of the First Central American Epidemics. *FAO Plant Protection Bulletin*, **24**, 36-42.

[19] Addinsoft (2020) XLSTAT Statistical and Data Analysis Solution. New York. https://www.xlstat.com

[20] Hemsley, A. and Gray (2005) Allelopathic Effects of Mexican Sunflower *Tithonia diversifolia* on Germination and Growth of Spider Plant (*Cleome gynandra* L.). *Journal of Biodiversity and Environmental Sciences*, **2**, 26-35.

[21] Umar, O.B., Alex, R.D. and Obukohwo, E.E. (2015) Phytochemical and Proximate Composition of *Tithonia diversifolia* (Hemsl.) A. Gray. *Annals Food Science and Technology*, **16**, 195-200.

[22] Muirhead, I.F. and Jones, D.R. (2000) Fungal Diseases of the Foliage. Sigatoka Leaf Spots. In: Jones, D.R., Ed., *Diseases of Banana*, *Abaca and Ensete*, CABI Publication, Wallingford, 544 p.

[23] Abadie, C., Hubert, O., Ngando, J.E., Ngoh, G., Mbeguie-A-Mbeguie, D., de Lapeyre de Bellaire, L. and Chillet, M. (2008) Evidence of the Effects of *Mycosphaerella* Leaf Spot Diseases on Fruit Quality. *Mem. XVIII ACORBAT Meeting*, Guayaquil, Ecuador, ACORBAT International, 8 p.

[24] Chillet, M., Abadie, C., Hubert, O., Chilin-Charles, Y. and de Lapeyre de Bellaire, L.

(2009) Sigatoka Disease Reduces the Greenlife of Bananas. *Crop Protection*, **26**, 1078-1082. https://doi.org/10.1016/j.cropro.2006.09.012

[25] Ewané, C.A., Lassois, L., Brostaux, Y., Lepoivre, P. and de Lapeyre de Bellaire, L. (2013) The Susceptibility of Bananas to Crown Rot Disease Is Influenced by Geographic and Temporal Effects. *Canadian Journal of Plant Pathology*, **35**, 27-36. https://doi.org/10.1080/07060661.2012.733731

[26] Mur, L.A.J., Simpson, C., Kumari, A., Gupta, A.K. and Gupta, K.J. (2016) Moving Nitrogen to the Centre of Plant Defence against Pathogens. *Annals of Botany*, **119**, 703-709. https://doi.org/10.1093/aob/mcw179

[27] Ewané, C.A., Ange, M.C., Felix, N.E. and Boudjeko, T. (2020) Influence of Clam Shells and *Tithonia diversifolia* Powder on Growth of Plantain PIF Seedlings (*var*. French) and Their Sensitivity to *Mycosphaerella fijiensis. African Journal of Agricultural Research*, **15**, 393-411. https://doi.org/10.5897/AJAR2019.14486

[28] Ewané, C.A., Meshuneke, A., Tatsegouock, R.N. and Boudjeko, T. (2020) Vertical Layers of *Tithonia diversifolia* Flakes Amendment Improves Plantain Seedling Performance. *American Journal of Agricultural Research*, **5**, 95.

[29] Meshuneke, A., Ewané, C.A., Tatsegouock, R.N. and Boudjeko, T. (2020) *Tithonia diversifolia* Mulch Stimulates the Growth of Plantain PIF Seedlings and Induces a Less Susceptibility to *Mycosphaerella fijiensis* in the Nursery. *American Journal of Plant Science*, **11**, 672-692. https://doi.org/10.4236/ajps.2020.115050